compared FTIR light-minus-dark ($P^+Q_A^-/PQ_A$) differential spectra in hydrated ($r = 76\%$) and dehydrated ($r = 11\%$) RC films over the 4000-1000 cm^{-1}. The spectra differ significantly in the 3750-3550 cm^{-1} range, the band attributed to weakly hydrogen bonded water molecules [5] being strongly reduced in the dried film. Dehydration also affects the 1800-1200 cm^{-1} range, which includes contributions from P, the quinones and the peptide. Optical absorption measurements performed under the same photoexcitation regime reveal a slow (t~5 s) kinetic component of $P^+Q_A^-$ recombination which disappears in the dehydrated sample, indicating at low *r* a destabilization of the charge separated state. As a whole the data suggest a correlation between the hydration shell dynamics and the conformational RC dynamics which stabilize the charge separated state.

References

[1] B.H. MacMahon, J.D. Muller, C.A. Wraight, U. Nienhaus, Biophys. J. 74 (1998) 2567–2587.
[2] G. Palazzo, A. Mallardi, A. Hochkoeppler, L. Cordone, G. Venturoli, Biophys. J. 82 (2002) 558–568.
[3] G. Katona, A. Snijder, P. Gourdon, U. Andréasson, Ö. Hansson, L.-E. Andréasson, R. Neutze, Nat. Struct. Mol. Biol. 12 (2005) 630–631.
[4] T. Iwata, M.L. Paddock, Okamura, H. Kandori, Biochemistry 48 (2009) 1220–1229.
[5] M. Malferrari, F. Francia, G. Venturoli, J. Phys. Chem. B 115 (2011) 14732–14750.

doi:10.1016/j.bbabio.2012.06.386

20P7

Molecular basis for semiquinone stabilization in respiratory enzymes: a pulsed EPR study of the menasemiquinone binding mode in *E. coli* nitrate reductase A

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Quinone binding sites (or Q sites) in respiratory complexes are the primary places for the production of reactive oxygen species that occurs as side reactions during the catalytic cycle and leads to cellular oxidative stress. This is mainly due to the transient formation of the highly reactive semiquinone species at these sites during electron transfer processes. Indeed, semiquinone stabilization is an obligate step during these processes since quinols/quinones are two-electron redox components while the proximal metal centers within respiratory complexes are one-electron transfer systems. The stabilization degree of a protein-bound semiquinone can differ by several orders of magnitude depending on the enzyme, and its importance for the function of the enzyme remains to be established. Moreover, the molecular determinants that drive this stability remain to be elucidated.

Due to the very high stability of the menasemiquinone bound to its quinol oxidation site (QD), *E. coli* nitrate reductase A (NarGHI) is a prime model for investigating the relationship between semiquinone binding mode and stabilization. Indeed, we have previously shown that this radical exhibits the highest stability measured so far for a quinone-utilizing respiratory enzyme [1]. Taking advantage from this peculiar property, the radical was used as a magnetic probe of its immediate environment. The detection of weak magnetic couplings between the unpaired electron and neighboring nuclei provided unprecedented information on the menasemiquinone binding mode [2, 3]. Combining multifrequency high-resolution pulsed EPR methods and H_2O/D_2O exchange experiments, several hydrogen atoms were unambiguously detected in the vicinity of the radical. They were assigned to specific chemical groups from either the quinone itself or from a single H-bond having unusual characteristics. Taken together, these results indicate a peculiar binding mode of the menasemiquinone at the NarGHI QD site which we consider to strongly contribute to its unusual redox properties.

References

[1] S. Grimaldi, P. Lanciano, P. Bertrand, F. Blasco, B. Guigliarelli, Biochemistry 44 (2005) 1300–1308.
[2] S. Grimaldi, R. Arias Cartin, P. Lanciano, S. Lyubenova, B. Endeward, T.F. Prisner, A. Magalon, B. Guigliarelli, J. Biol. Chem. 285 (2010) 179–187.
[3] S. Grimaldi, R. Arias-Cartin, P. Lanciano, S. Lyubenova, R. Szenes, B. Endeward, T.F. Prisner, B. Guigliarelli, A. Magalon, J. Biol. Chem. 287 (2012) 4662–4670.

doi:10.1016/j.bbabio.2012.06.387

20P8

Computational investigation of the electronic structure of the Cu_A site in bovine cytochrome *c* oxidases: the functional role of the axial methionine residue

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Cytochrome *c* oxidase (C*c*O), the terminal enzyme of the electron transport system, reduces an oxygen molecule, and thereby generates the gradient of the proton concentration between the matrix and the intermembrane space of mitochondria or the periplasmic space of bacteria. In the Cu_A site of C*c*O, two Cu ions form a covalent bond, and receive electrons from cytochrome *c*, thereby providing the electrons with heme *a*. The previous experiments revealed that the substitutions of the axial Met ligand, which coordinates to a Cu ion, with leucine largely induced the changes of the redox potentials in various species. For example, for *R. sphaeroides* C*c*O, the redox potential of the M207L mutant increases by 118 mV (large) from that of wild type. In contrast, for *T. thermophilus* ba_3 oxidase, the redox potential of the mutant increases by 53 mV (middle), and for the engineered azurin, which is a reconstructed azurin possessing a similar Cu-coordination in the Cu_A site, the redox potential of the mutant increases by 16 mV (small). Thus, since the effects of the Met residue on the mutants are likely to be different among the species, the functional roles of the Met residue are still ambiguous.

In this study, to explain this diversity, we theoretically investigated the electronic structures of the Cu_A site by employing hybrid *ab initio* quantum mechanics / molecular mechanics calculation. As a result of the analysis, we revealed that the Met residue creates the characteristic feature in the electronic structure of the Cu_A site, *without the significant rearrangements*. Furthermore, we calculated the inner-sphere reorganization energy of the Cu_A site with respect to the wild type and the mutants, and found that the effects of the Met residue are not significant. Accordingly, we concluded that the Met residue may act as the "fine-modulator" of the properties relevant to the various reactions occurring in C*c*O. Moreover, we investigated the